Docket No.: 04393/0202300-US0
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Kunihiro Ohta *et al.*

Application No.: 10/522,644 | Confirmation No.: 7488

Filed: February 28, 2005 | Art Unit: 1633

For: METHOD OF ENHANCING HOMOLOGOUS RECOMBINATION OF SOMATIC CELLS AND METHOD OF CONSTRUCTING SPECIFIC ANTIBODY | Examiner: M. G. Leavitt

DECLARATION OF HIDETAKA SEO, PH.D.
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Hidetaka Seo, hereby declare and state as follows:

1. I am a citizen of Japan and am more than twenty-one (21) years of age.

2. I received a Bachelor of Science in 1997, a Master's Degree in Biophysics and Biochemistry in 1999, and Doctorate of Philosophy in Biophysics and Biochemistry in 2003; all of my degrees are from the University of Tokyo. From 2003-2004, I was a Postdoctoral Fellow at the Small Business Promotion Public – Corporation Saitama Prefecture. I have been the Research and Development Director of Chiome Bioscience, Inc. since 2005 during which I have specialized in antibody generation and production. I also have authored or co-authored at least 8 scientific publications and review articles in peer-reviewed journals, most of which relate to the fields

of biology, genetics, and immunology, including antibody technology. A copy of my *curriculum vitae* is attached to this Declaration at Tab 1.

3. I am a named inventor in the above-identified patent application. As such, I am familiar with the specification and currently pending claims of U.S. Patent Application No. 10/522,644 ("the '644 Application") and the record of the USPTO proceedings involving the '644 Application. I have also reviewed the Final Office Action mailed July 25, 2008 and the prior art applied by the Examiner to reject the claims. I submit this declaration in support of the patentability of the present claims.

4. The invention described in this application pertains to methods of antibody production comprising enhancing DNA homologous recombination at an antibody locus when producing antibodies from chicken-derived B cells in which DNA homologous recombination occurs by relaxation, with a histone deacetylase inhibitor, of the chromatin structure of the chromosomes in the chicken-derived B cells, to obtain diverse antibodies.

5. I understand that the above-captioned application claims priority to a patent application, Japanese Application No. JP 2002-221232, which was filed in the Japan Patent Office on July 30, 2002. I also understand that the relevant time for evaluating obviousness of the current application's claims is the date when that priority application was filed (*i.e.*, July 30, 2002).

6. A person of ordinary skill in these arts around the time of July 30, 2002 would have been, for example, a person with a Ph.D. in a field of biology, genetics, or immunology, with a specialty in antibody technology and/or 1-2 years of experience in antibody generation or production. This person would have an understanding (to the extent available at the time as reflected

in the scientific literature) of different mechanisms for DNA recombination and antibody generation or production, as well as an understanding of how to enhance antibody production and/or diversity.

7. Homologous recombination is a defining attribute of gene conversion. In chicken-derived B cells, homologous recombination always involves gene conversion.

8. The processes of V(D)J recombination and homologous recombination are very different.

9. It is well accepted in the field (and was well accepted at the relevant time) that cells undergoing homologous recombination would not also be undergoing V(D)J recombination because homologous recombination and V(D)J recombination are different processes. *See* Grawunder et . al. (*Molecular Cell*, 1998, 2:477-484; copy enclosed at Tab 2), page 471, column 1, first full paragraph. ("There are two pathways for the repair of double-strand breaks. One is homologous recombination (HR), which occurs during late S and G2 of the cell cycle. The other pathway is nonhomologous DNA end joining (NHEJ). This is the predominant pathway during G0, G1, and early S phases of the cell cycle.") and page 477, column 1, Summary ("V(D)J recombination is a double-strand DNA breakage and rejoining process that relies on the NHEJ for the joining steps.").

10. Another difference is that "V(D)J recombination is a site-specific recombination process that occurs only in developing lymphocytes and only between Ig and T cell receptor gene segments flanked by conserved recombination signal sequences." *See* Bassing et al. (*Cell*, 2002, 109:S45-

S55; hereafter "Bassing"; copy enclosed at Tab 3), page S45, column 2, first full paragraph, first sentence. Additionally, "[t]he joining phase of the V(D)J recombination reaction is carried out primarily by ubiquitously expressed nonhomologous DNA end-joining proteins." *See* Bassing, page S45, column 2, first full paragraph, last sentence. On the other hand, in the mature lymphocytes of the current invention, DNA breaks are repaired by homologous recombination.

11. Another difference between V(D)J recombination and gene conversion is that V(D)J recombination is a DNA sequence-specific event (*See* supra ¶10). In contrast, gene conversion is not a sequence specific event.

12. In gene conversion, unlike in V(D)J recombination, DNA loci are not deleted. Gene conversion permits repeated and continuous homologous recombination because donor pseudo-V genes used in gene conversion do not undergo deletions. *See* Arakawa and Buerstedde (*Developmental Dynamics*, 2004, 229:458-464; "Arakawa"; copy enclosed at Tab 4), p. 460, first column, first full paragraph ("During B-cell development in the bursa, segments of pseudogene sequences appear in the rearranged V gene segments, but the pseudogene donor sequences do not change.") and Figure 2. Since DNA is not deleted during gene conversion, the full repertoire of DNA is accessible for undergoing homologous recombination thereby maximizing the diversity of antibodies generated.

13. On the other hand, genomic DNA loci often undergo deletion during V(D)J recombination. *See* Bassing, p. S45, column 2, first full paragraph ("V(D)J recombination is initiated via introduction of DNA double-strand breaks (DSBs) between the V, D, and, J segments and flanking RSs [recombination signals]; subsequently, RS ends are precisely joined, while

coding ends are modified via a process that involves potential nucleotide loss and potential nucleotide addition."). Due to deletion of genomic DNA, less DNA is available to undergo V(D)J recombination thereby limiting the diversity of antibodies that may be generated.

14. Agata et al. (*J. Exp. Med.*, 2001, 193(7):873-879; hereafter "Agata"; copy enclosed at Tab 5), provides data showing that achieving high levels of recombination events in V(D)J recombination is limited. Specifically, in Agata, a mild increase of only several percent in recombination events was observed. *See* Agata, Fig. 2A. This increase in the number of recombination events reported by Agata is not adequate to establish a diversity of antibodies or a library of diverse antibodies. Therefore, at the relevant time, Agata highlights the poor ability of V(D)J recombination to lead to high levels of antibody diversity.

15. Because V(D)J recombination and homologous recombination are different processes, teachings applicable to V(D)J recombination are not applicable to homologous recombination. This is shown more specifically in paragraphs 16-18 below.

16. McMurry states that hyperacetylation would be beneficial in V(D)J recombination. However, McMurry does not make statements applicable to gene conversion, a process that instead proceeds via homologous recombination. Furthermore, McMurry does not teach using a histone deacetylase inhibitor.

17. The reference Agata established why the statements of McMurry were not applicable to homologous recombination. For example, Agata achieves activation of V(D)J recombination with trichostatin A (TSA), which is a

histone deacetylase inhibitor, at inactive V gene loci, but little V(D)J recombination at active V gene loci. Specifically, TSA activated V(D)J recombination at the Vγ3 locus, which is naturally silent and inactive in V(D)J recombination. TSA had little effect at the Vγ2 locus, which is normally active in V(D)J recombination. *See* Agata, page 876, column 2, last paragraph ("By TSA treatment, however Vγ3 but not Vγ2 coding joints were significantly increased in adult-derived cells (5- and 17-fold on day 10 and 14, respectively compare TSA + to – lanes in Fig. 2B).")

18. In homologous recombination, V gene loci are active in homologous recombination. Therefore, the reason why Agata added trichostatin A in V(D)J recombination, to activate inactive loci, does not exist in homologous recombination.

19. At the relevant time, the mechanism of gene conversion was unknown. Specifically, the mechanism of action of AID, which is required in gene conversion, was unknown, as explained in more detail in paragraphs 20-23 below.

20. There were two main hypotheses discussed in the art at the relevant time of filing and at least up to February 2004. *See* Arakawa, page 462, bottom of first column to page 462, middle of second column ("At the moment, two alternative models are proposed to explain the induction of recombination and hypermutation by AID.") and Table 3 on page 462.

21. One hypothesis would have AID modify DNA directly ("DNA editing model"). Specifically, AID would catalyze cytosine deamination resulting in guanine/uracil (G/U) mismatches.[1] *See* Arakawa, page 462, end of first

[1] This is what in fact occurs.

column ("According to the first model AID catalyzes cytosine deamination leading to guanine/uracil (G/U) mismatches (DNA editing model, Fig. 3A) . . . .").

22. The other hypothesis would have AID modify messenger RNA (mRNA) encoding DNA-modifying enzymes ("RNA editing model"), which leads to the translation of an active protein that then introduces a DNA alteration in the rearranged V gene segment. *See* Arakawa, page 462, bottom of first column to page 462, beginning of second column ("[I]n the second model AID edits an mRNA that encodes the DNA-modifying activity (RNA editing model, Fig. 3B).").

23. The effect of a histone deacetylase inhibitor like TSA in cells undergoing gene conversion had different implications depending on the hypothesis followed, and, even within each known hypothesis, the effect of TSA was uncertain.

24. With respect to the DNA editing model, the ability of a histone deacetylase inhibitor to enhance accessibility of DNA in cells undergoing gene conversion to AID was unpredictable. One could argue that it was plausible that a histone deacetylase inhibitor could have resulted in accelerated DNA deamination followed by enhanced gene conversion frequency. On the other hand, it was as plausible that a histone deacetylase inhibitor could have had the undesirable results of up-regulation of inhibitors of AID and down-regulation of homologous recombination factors leading to inhibition of gene conversion.

25. With respect to the RNA editing model, the effect of a histone deacetylase inhibitor was also unpredictable and in fact might have interfered with or

inhibited AID activity. With this hypothesis, a histone deacetylase inhibitor would have affected RNA, not DNA directly, and it was possible that a histone deacetylase inhibitor could have altered expression of DNA-modifying enzymes negatively or positively. There are numerous enzymes involved in homologous recombination that could have been upregulated or downregulated. Such altered expression could have enhanced or impaired gene conversion. Hence, it was unpredictable whether or how a histone deacetylase inhibitor would have altered the expression pattern of DNA-modifying enzymes and whether or how the modified expression would impact gene conversion, a process that proceeds via homologous recombination.

26. I and my co-inventors have discovered that by treating chicken-derived B cells with TSA, it is possible to obtain an extreme increase in homologous recombination (e.g., at least 2 orders of magnitude), which is only now known to proceed by the DNA editing model.

27. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code, and that such willful false statements may jeopardize the validity of this application or of any patent issuing therefrom.

Respectfully submitted,

Dated: June 15, 2009

Hidetaka Seo, Ph.D.

Attachments:

Tab 1: *Curriculum vitae* of Dr. Hidetaka Seo

Tab 2: Grawunder et . al. (*Molecular Cell*, 1998, 2:477-484)

Tab 3: Bassing et al. (*Cell*, 2002, 109:S45-S55)

Tab 4 Arakawa and Buerstedde (*Developmental Dynamics*, 2004, 229:458-464)

Tab 5 Agata et al. (*J. Exp. Med.*, 2001, 193(7):873-879)